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Unexpected oocyst shedding by cats fed *Toxoplasma gondii* tachyzoites: In vivo stage conversion and strain variation

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Abstract

Tachyzoites, bradyzoites (in tissue cysts), and sporozoites (in oocysts) are the three infectious stages of *Toxoplasma gondii*. The prepatent period (time to shedding of oocysts after primary infection) varies with the stage of *T. gondii* ingested by the cat. The prepatent period (pp) after ingesting bradyzoites is short (3–10 days) while it is long (18 days or longer) after ingesting oocysts or tachyzoites, irrespective of the dose. The conversion of bradyzoites to tachyzoites and tachyzoites to bradyzoites is biologically important in the life cycle of *T. gondii*. In the present paper, the pp was used to study in vivo conversion of tachyzoites to bradyzoites using two isolates, VEG and TgCkAr23. *T. gondii* organisms were obtained from the peritoneal exudates (pex) of mice inoculated intraperitoneally (i.p.) with these isolates and administered to cats orally by pouring in the mouth or by a stomach tube. In total, 94 of 151 cats shed oocysts after ingesting pex. The pp after ingesting pex was short (5–10 days) in 50 cats, intermediate (11–17) in 30 cats, and long (18 or higher) in 14 cats. The strain of *T. gondii* (VEG, TgCKAr23) or the stage (bradyzoite, tachyzoite, and sporozoite) used to initiate infection in mice did not affect the results. In addition, six of eight cats fed mice infected 1–4 days earlier shed oocysts with a short pp; the mice had been inoculated i.p. with bradyzoites of the VEG strain and their whole carcasses were fed to cats 1, 2, 3, or 4 days post-infection. Results indicate that bradyzoites may be formed in the peritoneal cavities of mice inoculated intraperitoneally with *T. gondii* and some bradyzoites might give rise directly to bradyzoites without converting to tachyzoites.
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Keywords: *Toxoplasma gondii*; Tachyzoites; Bradyzoites; Oocysts; Cats; In vivo stage conversion

1. Introduction

Tachyzoites and bradyzoites are structurally and biologically different stages of *Toxoplasma gondii* found in tissues of infected animals and humans (Dubey et al., 1998). Post-natally, humans or animals become infected with *T. gondii* by ingesting food or water contaminated with oocysts from infected cat feces or by ingesting tissue cysts from uncooked and undercooked infected meat (Dubey and Beattie, 1988). After ingestion, sporozoites or bradyzoites convert to tachyzoites inside the host tissues (Dubey, 1997; Dubey et al., 1997). Tissue cysts begin to form

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the first week p.i. and are thought to persist for life (Dubey and Frenkel, 1976). Reactivation of latent infection, for example, in patients with acquired immunodeficiency syndrome (AIDS) can lead to fatal toxoplasmosis. Reactivation is thought to be due to rupture of tissue cysts and formation of new tachyzoites and bradyzoites. It is uncertain whether bradyzoites from older tissue cysts can directly give rise to new tissue cysts or have to go though the tachyzoite stage first. Bradyzoites are less susceptible to chemotherapy that is effective against tachyzoites. Therefore, the fate of bradyzoites in host tissues is of clinical significance. Recent in vitro studies indicate that bradyzoites may give rise to bradyzoites without first converting into tachyzoites (Weiss et al., 1995; Dzierszinski et al., 2004).

There has been great interest in studying conditions needed for stage conversion of *T. gondii* (Weiss et al., 1995; Frenkel, 1996; Gross et al., 1996; Bohne et al., 1996; Jerome et al., 1998; Dubey et al., 1998; Dzierszinski et al., 2004). Structurally, tachyzoites have a centrally located nucleus, have a few or no PAS-positive granules, and are found during acute infection. Bradyzoites have a terminally located nucleus, have many PAS-positive granules, are enclosed in a resistant tissue cyst wall, and are more prevalent during the chronic stage (Dubey and Frenkel, 1976). Several monoclonal antibodies specific for tachyzoites and bradyzoites and genetic markers have been used to investigate the transition (Weiss et al., 1995; Bohne et al., 1996; Dzierszinski et al., 2004). However, the transition stage between tachyzoites and bradyzoites and vice versa is not well defined structurally or antigenically (Frenkel, 1996; Dubey et al., 1998).

Biologically, bradyzoites are resistant to gastric digestion and thus are infectious orally whereas tachyzoites are destroyed by gastric juice (Jacobs et al., 1960). However, recent studies indicate that susceptibility to acid-pepsin digestion is not a reliable criterion to distinguish bradyzoites from tachyzoites because occasionally tachyzoites survived pepsin digestion and tachyzoites were infective orally to mice and cats (Dubey, 1998a). Cell culture is not a reliable method to obtain pure culture of tachyzoites because bradyzoites develop in cell culture (Hoff et al., 1977); further, a proportion of bradyzoites are thought to divide directly into bradyzoites, without conversion to tachyzoites (Weiss et al., 1995). One method to distinguish tachyzoites from bradyzoites is by bioassay in cats using prepatent period (pp) to oocyst shedding as a criterion. Cats fed bradyzoites shed oocysts with a pp of 3–10 days, irrespective of the dose or the strain of *T. gondii* (Dubey and Frenkel, 1972, 1976; Dubey, 2001) whereas those fed tachyzoites of the M-7741 strain shed oocysts with a pp of 19 days or longer. These latter observations were based on feeding cats tissues of mice that had been infected up to 2 days after systemic inoculation with any stage of *T. gondii* (Dubey and Frenkel, 1976). Cats fed tachyzoites from the peritoneal exudate (pex) shed oocysts with a long pp (>19 days). These observations indicated that bradyzoites are not found in pex. In the present paper, I report shedding of oocysts by cats fed pex from mice infected with two other isolates (VEG and TgCKAr23) of *T. gondii* suggesting the presence of bradyzoites in pex.

2. Materials and methods

2.1. Strains of *T. gondii*

The VEG strain of *T. gondii* was isolated from the blood of an AIDS patient (Dubey et al., 1996). Its early maintenance history is unknown. Since 1995 it has been maintained in this laboratory by tissue cyst–oocyst passage. It has been used extensively to study the biology of toxoplasmosis in mice after oral infection with bradyzoites and oocysts (Dubey, 1997; Dubey et al., 1997). It is a type III strain using the classification of Howe and Sibley (1995) and is mildly pathogenic to mice.

The TgCkAr23 isolate was obtained from an asymptomatic chicken from Argentina in September 2004 (Dubey et al., 2005). It is a type I strain and is virulent for Swiss Webster (SW) mice. The original passage of the strain was used in this study. Five SW mice were inoculated subcutaneously (s.c.) with brain homogenate of this chicken. All five mice became sick and died or were euthanized on days 19, 19, 35, 35, and 35 post-inoculation (p.i.). Tachyzoites were seen in the lungs of mice at day 19 p.i. and tissue cysts were seen in the brains of mice at 35 day p.i.; these tissue cysts were used to obtain tachyzoites and oocysts in the present study.

2.2. Propagation of *T. gondii*

Tachyzoites were obtained from the peritoneal cavities of mice. Most isolates of *T. gondii*, including the VEG and the TgCkAr23, grow slowly in the peritoneal cavity of mice and it is difficult to obtain large numbers of tachyzoites of these strains unless mice are immune-compromised. Different procedures were used to immune suppress mice (Table 1). Initially, SW mice were immunosuppressed by one of the three methods: 2.5 mg cortisone acetate, twice weekly before and after *T. gondii* inoculation; 4 mg methyl

Table 1
Oocyst shedding by cats administered peritoneal exudates of mice infected with the *T. gondii* VEG strain

Trial no.	Inoculum			Mode of administration	No. of shed oocysts/no. of cats fed	Prepatent period (days)
	Mice and stage[a]	Passage no. for *T. gondii*	Day pex removed from mouse			
1	UC[b]	1	6	p.o.	2/2	17, 17
2	UC[c]	1	5	p.o.	2/2	8, 9
3	UC[c]	1	5	p.o.	7/8	7, 7, 7, 7, 13[d], 15[d], 16[d]
4	UC[c]	2	5	p.o.	6/8	6, 6, 8, 9, 14, 16
5	UC[e]	2	3	p.o.	4/4	5, 5, 5, 5
6	PEC[f]	1	5	p.o.	2/2	5, 6
		1	7	p.o.	1/1	5
		2	7	p.o.	1/1	5
		3	5	p.o.	1/1	22
		4	6	p.o.	0/1	N/A
		6	3	p.o.	1/2	13
		7	3	p.o.	1/4	19
		8	5	p.o.	0/2	N/A
		10	3	Stomach tube	1/13[g]	21
7	PEC, FB[f]	1	4	p.o.	2/2	6, 9
		2	4	p.o.	1/4	12
		3	5	p.o.	2/2	7, 10
		4	5	p.o.	4/5	6, 7, 7, 9
		5	7	p.o.	2/2	8, 10
		6	3	p.o.	3/3	6, 7[d], 19[d]
		7	4	p.o.	1/2	5[d]
		8	3	p.o.	2/2	6[d], 6[d]
8	PEC, FB[f]	1	4	p.o.	2/2	4, 24
		2	4	p.o.	1/2	27
		3	4	p.o.	1/1	14
9	PC[e,f]	1	5	Stomach tube	8/12	10, 11, 15, 17, 17, 18, 21, 21, *N*, *n*, *n*, *n*
				p.o.	3/12	11, 16, 21, *N*, *N*, *N*, *N*, *N*, *N*, *n*, *n*, *n*
10	Oocysts[e,f]	1	5	p.o.	8/8	10, 12, 12, 12, 13, 13, 13, 14
		2	5	p.o.	4/4	5, 5, 5, 5

n: Oocysts not shed; *N*: Oocysts not shed, but cats developed antibody titers of 1:400 or higher day 23 p.i.
[a] UC, untreated tissue cysts; PC, pepsin-treated tissue cysts; PEC, Percoll cleaned tissue cysts; FB, filtered bradyzoites.
[b] SW mice, prednisolone acetate treated.
[c] SW mice, cortisone treated.
[d] Numbers of oocysts shed by these eight cats were compared.
[e] KO mice.
[f] SW mice, dexamethasone treated.
[g] Six cats developed *T. gondii* MAT titer $\geq$1:800 day 21 p.i.

prednisolone acetate (UpJohn, Kalamazoo, MI) once before *T. gondii* inoculation; or 1 mg/ml of dexamethasone in drinking water (Sigma, St. Louis, MO) from the day of *T. gondii* inoculation and continuing until the end of the experiment, or interferon gamma gene knockout (KO) mice were used. The KO mice are highly susceptible to intracellular parasites, including *T. gondii* (Dubey and Lindsay, 1998).

To obtain tachyzoites from *T. gondii* tissue cysts, brains of mice inoculated 1–2 months previously with *T. gondii* were homogenized with a pestle and mortar in a 0.9% NaCl solution (saline) and the homogenate was inoculated i.p. into immune-compromised mice. In certain trials, mice were inoculated with free bradyzoites (Table 1). To release bradyzoites from tissue cysts, mouse brains containing tissue cysts were incubated in pepsin solution, then neutralized with 1.2% sodium bicarbonate (Dubey, 1998b). To obtain clean bradyzoites, tissue cysts were separated from brain in a Percoll gradient (Cornelissen et al., 1981), washed, and bradyzoites were released by acid-pepsin treatment (Popiel et al., 1996). After washing, the bradyzoite suspension was passed through a 3-μm membrane filter (Nuclepore, Pleasanton, CA).

To obtain tachyzoites from sporozoites, oocysts were produced in cats, sporulated in 2% sulfuric acid, washed, and inoculated into mice i.p. with or without treatment with 5.25% sodium hypochlorite (Dubey and Beattie, 1988). Peritoneal cavities of mice inoculated with *T. gondii* were gavaged with saline and the aspirate was examined microscopically to ascertain the presence of *T. gondii*. Organisms so obtained were considered passage 1; organisms from passage 1 were either inoculated into cats or into mice; up to 10 passages were made in mice (Table 1). The subpassaged lines were later discontinued.

2.3. Bioassay in cats for *T. gondii*

To identify stages of *T. gondii* in the inoculum, materials were bioassayed in *T. gondii*-free cats from a closed colony. The management of cats, method of feces collection, and oocyst enumeration were described in detail (Dubey, 1995). All experiments were performed as per the guidelines of the U.S. Department of Agriculture Animal Care Committee. For bioassay, cats were fed either whole mice or given a tachyzoite suspension. Suspensions of *T. gondii* (1–5 ml) were poured into the mouth by holding of the cat head in an upward position or were deposited directly in the stomach of anaesthetized cats by a stomach tube (Dubey, 2002). Antibodies to *T. gondii* were determined in sera of cats by using the modified agglutination test incorporating whole formalin-fixed *T. gondii* tachyzoites as described (Dubey and Desmonts, 1987); antibodies to *T. gondii* were not found in 1:25 dilution of serum of any of the 159 cats used in this study.

Feces of each cat were examined microscopically for oocysts. For this, the entire sample was emulsified with a small volume of water, and then ~10 g were mixed with ~40 ml of a sucrose solution (specific gravity, 1.18), filtered through gauze, and centrifuged in a 50-ml tube at 2000 rpm (~1200 × *g*) for 10 min. A drop of the float from the meniscus was examined microscopically for oocysts. If oocysts were detected, the entire daily sample was mixed with sucrose solution in 50-ml tubes and centrifuged at 2000 rpm for 10 min. Then supernatant in the 50-ml tube was mixed with 200 ml of water and centrifuged. The supernatant was discarded, the sediment was mixed with water, and all samples from each cat for each day were pooled, centrifuged, and finally suspended in water to make a final volume of 100 ml. Oocysts were counted in the four WBC chambers of a hemacytometer. The number of oocysts in four WBC chambers was multiplied by 2500 × 100 (total volume). Thus, the sensitivity of counting oocysts was 250,000 per fecal sample. Oocysts or fecal floats were bioassayed in mice and the prepatent periods were based on infectivity in mice (Dubey, 2001).

2.4. Experiment 1

In this experiment, shedding of oocysts by cats fed tissues of whole mice infected with the VEG strain was investigated. Tissue cysts separated from the brains of 40 mice infected orally with VEG oocysts 5 months earlier were washed and inoculated i.p. into 40 mice. On days 1–4 after inoculation, three to four mice were killed, skinned, and fed to two cats for each day. Feces of cats were examined for oocysts shedding.

2.5. Experiment 2

In this experiment involving 10 trials and 113 cats shedding of oocysts by cats fed pex of the VEG strain was studied (Table 1). In the first nine trials, infections were initiated by i.p. inoculation of mice with bradyzoites. In trial no. 10 mice infections were initiated by i.p. inoculation with oocysts (Table 1). In trial no. 6, tachyzoites were subpassaged in mice for up to 10 passages and cats were administered tachyzoites from the 1–4, and 6–10 passages; thereafter, this line was discontinued. In trial no. 7, eight serial passages were made in mice and organisms from each passage were administered to cats (Table 1). In the remaining trials, only one to three passages were made.

To compare the effect of the mode of administration of pex on oocysts shedding in cats, in trial no. 8, pex obtained from mice was divided into two aliquots; one aliquot was poured in the mouths of 12 cats and the other aliquot was given by a stomach tube to additional 12 cats.

2.6. Experiment 3

In this experiment, shedding of oocysts by cats fed pex of the TgCkAr23 isolate of *T. gondii* was investigated. Cats were given pex (16 by mouth and 6 by stomach tube) derived from bradyzoites and 16 cats were fed pex derived from oocysts (Table 2).

3. Results

3.1. Experiment

One of two, one of two, two of two, and two of two (in total six of eight) cats fed mice infected i.p. with bradyzoites 1, 2, 3, or 4 days earlier, respectively, shed oocysts with a prepatent period of 5–8 days.

3.2. Experiment 2

In this experiment, 73 of 113 cats fed pex shed oocysts (Table 1). Prepatent periods were short (5–10 days) in 40 cats, intermediates (11–17 days) in 23 cats, and long (18 days or higher) in 10 cats.

The route of administration (by mouth versus by stomach tube) and the source of tachyzoites (bradyzoites versus sporozoites) did not affect the prepatent period (Table 1). The day (3–7) pex was removed from the mice did not affect the prepatent period (Table 1).

Cats shed millions of oocysts after ingesting tachyzoites from pex and peak oocyst shedding occurred 1–3 days after oocysts were first seen in feces. The number of oocysts shed by four cats with prepatent periods of 13, 15, 16, and 19 days were 88, 7.5, 349, and 37 million, respectively. The number of oocysts shed by four cats with a prepatent period of 5 or 6 days was 227, 50, 118, and 329 million; these cats had been fed pex from the seventh and eighth passages (Table 1).

3.3. Experiment 3

Twenty one of 38 cats administered pex of the TgCkAr23 isolate of *T. gondii* shed oocysts (Table 2). The prepatent periods were short (5–10 days) in 10 cats, intermediate (11–17 days) in 6 cats, and long (18 days or higher) in 5 cats. The prepatent periods in 4 of the 12 cats that were fed pex from mice inoculated i.p. with oocysts were 15, 17, 19, and 22 days (Table 2).

Table 2
Mode of infection, source of inoculum, and shedding of oocysts by cats fed pex from mice inoculated with the the TgCkAr23 isolate of *T. gondii*

Trial no.	Inoculum			Mode of infection	No. of cats shed oocysts/no. fed	Prepatent period (days)
	Source	Passage no.	Day pex removed			
11	Free bradyzoites[a]	1	5	p.o.	4/6	12, 17, 20, 24, *n*, *n*
			4	Stomach tube	2/6	10, 10, *n*, *n*, *n*, *n*
		2	5	p.o.	9/10	7, 7, 8, 9, 9, 10, 10, 11, 21, *n*
12	Oocysts, KO mice	1	7	p.o.	4/12	15, 17, 19, 22, *N*, *N*, *N*, *N*, *N*, *N*, *N*, *N*
		2	5	p.o.		8, 13, *N*, *N*

n: Oocysts not shed; *N*: Oocysts not shed, but cats developed antibody titers of 1:400 or higher day 23 p.i.
[a] Pepsin-treated bradyzoites were inoculated i.p. into KO mice and dexamethasone-treated SW mice.

4. Discussion

All published studies indicate that the prepatent period in cats is short (3–10 days) after ingesting bradyzoites and long (18 days or more) after ingesting oocysts and this does not vary with strain of *T. gondii* (Table 4). The discrepancy in results in the studies summarized in Tables 3 and 4 is related to infections induced by tachyzoites or acutely infected mice. Reasons for this variability in pp after ingesting tachyzoites may include *T. gondii* strains, parasite stages used, dose, history of strains, sources, and methods used to obtain tachyzoites.

Until now, organisms obtained from pex of mice were considered to be tachyzoites and one criterion for this assumption was based on feeding pex to cats using the M-7741 and Me-49 strains of *T. gondii* (Table 3). Results in the present study are different than those reported previously. In the present study, 50 cats shed oocysts with a short prepatent period after ingesting *T. gondii* from pex of mice whereas the prepatent period was long (19 days or more) after feeding tachyzoites; pex was the source of inocula for both studies. In one experiment of Dubey and Frenkel (1976), tachyzoites from pex were directly inoculated into intestinal lumen of cats; five of six cats given tachyzoites shed oocysts with prepatent periods of 21–34 days (Table 4). In a follow up study, 10 additional cats were inoculated directly in the intestine with tachyzoites of the M-7741 strain and then killed 1–25 days later for histologic and fecal examinations; none of them shed oocysts before 23 days (Dubey, 2002). Similar results were obtained with the Me-49 strain with one exception (Dubey, 2002). Of 26 cats fed tachyzoites of the Me-49 strain and observed for at least 3 weeks, 12 cats shed oocysts with a long prepatent period (19 days or more) and 1 cat

Table 3
Oocyst shedding by cats fed mice inoculated with different stages of different isolates of *T. gondii*

T. gondii strain	Infection in mice			No. of cats shed/no. of cats fed	Prepatent period	Reference
	Stage inoculated	Routes	Day of infection			
M-7741	Pex (tachyzoites)	Several	1	1/6	40	Dubey and Frenkel (1976)
			2	3/18	26, 26, 27	
			3	4/19	6, 8, 37, 37	
			4	8/10	4–7	
			5	11/13	3–6	
			6	8/8	3–6, 44	
			7–30	30/30	3–6	
	Bradyzoites	Several	2–6	0/16	NA	
			7	1/8	7	
			9	3/4	4–7	
			11	2/2	4 or 5	
	Oocysts	Several	2	0/2		
			5–7	0/21	NA	
			9	3/4	6–9	
			11	4/4	4 or 5	
Me-49, GT-1, PT-89	Bradyzoites	p.o.	1	0/3	NA	Dubey (1997)
			2	0/1	NA	
			4	0/1	NA	
			6	1/1	7	
VEG	Oocysts	p.o.	4	0/1		
			6	0/1		
			7	1/1	7	
	Bradyzoites	p.o.	1	0/4	NA	Dubey (1997)
			2	0/2	NA	
			5	0/2	NA	
			6	2/2	7	

Table 4
Oocyst shedding by cats fed different stages and four different isolates of *T. gondii*

T. gondii strain	Stage fed	No. of cats shed oocysts/no. of fed	Prepatent period	Reference
M-7741	Tachyzoites (pex)[a]	5/6	21–34	Dubey and Frenkel (1976)
	Bradyzoites	199/204	3–8	
	Oocysts	15/134	21–41	Freyre et al. (1989) and Dubey (1996)
Me-49	Tachyzoites (pex)	12/26	5 (1 cat), 19–30 (11 cats)	Dubey (2002)
	Bradyzoites	10/10	5–7	Dubey (unpublished)
	Oocysts	Not done		
VEG	Bradyzoites (1–1000)	12/28	4–7	Dubey (2001)
	Oocysts (100–10,000)	8/12	18–35	Dubey (1996)
	Tachyzoites	72/148	5–18 or more	Present study
TgCkAr23	Tachyzoites	21/38	5–22	Present study

[a] Directly inoculated into intestinal lumen of cats.

shed oocysts with a short prepatent period of 5 days (Dubey, 2002).

One reason for the variability in these results could be due to the origin, and maintenance of the *T. gondii* strains. The M-7741 and Me-49 strains were obtained in 1958 from muscles of sheep and had been maintained in mice for many years before their use in life cycle studies that started in 1968. The M-7741 strain was used extensively to study the endogenous development and life cycle of *T. gondii* in cats after feeding tissue cysts (Dubey and Frenkel, 1972, 1976), oocysts (Freyre et al., 1989; Dubey, 1996), and tachyzoites (Dubey and Frenkel, 1976); this strain was lost and no longer available. Unfortunately, M-7741 is the only strain whose complete life cycle in cats is known. Only the tachyzoite cycle was studied with the Me-49 isolate. Studies using these three isolates (M-7741, Me-49, and VEG) have the drawback that they had been maintained in the laboratory for long time and this might have affected the results. Therefore, the recently obtained TgCkAr23 isolate was included in the present study and results were similar to those obtained with the VEG strain.

T. gondii strains isolated from animals or humans are usually maintained in the laboratory by passage in cell culture or animals and frequent passage in mice can change their biological properties. The ability to form oocysts was lost in the M-7741 strain by 30–35 rapid passages in mice (Frenkel et al., 1976) and in the GT-1 strain by 40 passages in cell culture (Lindsay et al., 1991). In addition, three other isolates (RH, Beverley, and AJH) that had been passed in mice for many years have lost the capacity to form oocysts (Frenkel et al., 1976). Therefore, the VEG strain has been maintained in my laboratory by tissue cyst–oocyst cycle and not passaged frequently in mice; each new trial or experiment was initiated with lines proven to produce oocysts. All lines of the VEG strain passaged more than twice in mice without going through the oocyst cycle were discarded. In the present study, in trial no. 7 cats fed pex removed from mice up to eighth passage induced oocyst shedding with a short prepatent period. However, in trial no. 6 only 1 of the 15 cats fed pex from the 8th and 10th passages shed oocysts and the prepatent period was long. These data suggest that even as few as eight passages in mice might alter the biological properties of *T. gondii*.

In the present study, mice were infected with *T. gondii* stages using the i.p. route; whether the route of inoculation affected the results is unknown, but unlikely. Dubey and Frenkel (1976) inoculated mice with tachyzoites of the M-7741 strain by four routes simultaneously (i.p., s.c., intramuscular, and intracerebrally) and then fed mice to cats. Of the 24 cats fed mice infected with tachyzoites 2 days previously, 4 cats shed oocysts with prepatent periods of 27–40 days. Of the 19 cats fed mice infected for 3 days, 4 shed oocysts, 2 with a short prepatent period (6 and 8 days) and 2 with a long prepatent period of 37 days. Of the 10 cats fed mice infected for 4 days, 8 shed oocysts with a short prepatent period (4–7 days). Based on these findings, it was proposed that the short prepatent period was related to the ingestion of bradyzoites and the long prepatent (>19 days) was related to the ingestion of tachyzoites. In addition, feeding of mice systemically inoculated with sporozoites or bradyzoites 6 days before feeding to cats, none of the 32 cats shed oocysts with a short prepatent period (Table 4). Similar results were obtained using the Me-49, P89, GT-1, and the VEG strains of *T. gondii* in cats fed mice orally infected with bradyzoites or oocysts (Dubey, 1997; Dubey et al., 1997). When mice were fed bradyzoites or oocysts of these four isolates (Me-49, GT-1, P89, and VEG) and bioassayed in cats 2–5 days after infection the cats did not shed oocysts within 10 days. Therefore, the results in the present study were unexpected. Also, unexpected were the prepatent periods of 11–17 days because in previous studies the longest prepatent period after feeding bradyzoites was 10 days.

The possible explanations for short prepatent period in cats fed pex of the VEG and the TgCkAr23 isolates in the present report are: (1) a few bradyzoites from the inoculum survive in the peritoneal cavity of mice. However, this is not applicable to infections initiated via oocysts and sporozoites and persistence of this phenomenon after several passages in mice. (2) Bradyzoites are produced in cells lining the peritoneal cavity of mice and are shed in the peritoneal cavity, or (3) tachyzoites are converted directly to bradyzoites in cat intestine and then induce the bradyzoite cycle; this was not confirmed histologically in cats fed tachyzoites of the Me-49 and M-7741 strains (Dubey, 2002). These results could be explained if we assume that occasionally bradyzoites may give rise to bradyzoites directly without converting to tachyzoites and this might differ with *T. gondii* strains and the intermediate (11–17 days) prepatent period is related to the transitional stage between tachyzoite and bradyzoite. With the use of the bradyzoite- (BAG1) and tachyzoite- (SAG1) specific markers, a few bradyzoites were found to give rise to bradyzoites in cell cultures without converting to tachyzoites (Weiss et al., 1995; Dzierszinski et al., 2004). However, in vivo this occurrence must be rare because in mice fed bradyzoites the BAG1 staining was lost 2 days p.i. and did not appear again until day 6 p.i. and these results were confirmed by bioassay in cats (Dubey, 1997). The bioassay in cats is very objective and the most sensitive because cats shed oocysts even after ingesting a few bradyzoites (Dubey, 2001). In two previous studies, mice fed bradyzoites or oocysts of five isolates (M-7741, Me-49, GT-1, P89, and VEG) and bioassayed in cats 2–5 days after infection the cats did not shed oocysts within 10 days, indicating that there were no bradyzoites in the inocula (Dubey and Frenkel, 1976; Dubey, 1997). In experiment 1 of the present study, however, two of four cats that were fed mice inoculated i.p. with bradyzoites shed oocysts with a short pp, indicating that either bradyzoites gave rise to bradyzoites directly or a few bradyzoites from the inocula had not yet converted to tachyzoites. It will be difficult or impossible to follow this change in vivo because of the rarity of this occurrence. It is not possible to estimate the number of bradyzoites in the inoculum by oocyst shedding in cats because cats can shed millions of oocysts by ingesting less than 10 bradyzoites (Dubey, 2001). Therefore, the number of oocysts shed by cats fed pex in the present study was not helpful in estimating the number of bradyzoites present in pex. Oocyst shedding by cats fed pex derived from different *T. gondii* passages varied. For example, none of the 15 cats fed pex from 8 or 10 passages (trial no. 6, experiment 2) shed oocysts with a short prepatent period whereas both cats fed pex of eighth passage from another line (trial no. 7, experiment 2) shed oocysts with a short prepatent period.

The transition of bradyzoite to tachyzoite and tachyzoite to bradyzoite is not an all or none phenomenon and the transitional stage has not been morphologically and biologically characterized. For example, tachyzoites lack PAS-positive granules that are numerous in bradyzoites and their synthesis and accumulation is gradual (Dubey and Frenkel, 1976). The intermediate prepatent periods (11–17 days) found in the present study may be related to the transitional stage between tachyzoites and bradyzoites. The prepatent period data suggest that all three stages may be present simultaneously in the pex and it will be biologically difficult to determine their relative numbers. For example, in trial nos. 3 and 8 (experiment 2) of the numerous organisms in the pex fed to cats, only a few were likely to be bradyzoites and intermediate stage because the same inocula gave rise to short, intermediate, and long prepatent period, assuming that each cat fed one bradyzoite can shed oocysts.

The life cycle of *T. gondii* in cats (definitive host) and in mice (intermediate host), and in cell culture varies. Cats are the only definitive hosts whereas all warm-blooded hosts, including the cat are intermediate hosts. *T. gondii* is more biologically adapted to transmission by bradyzoites (carnivorism) in cats by oocysts (fecal transmission) in intermediate hosts (Frenkel et al., 1970; Dubey, 2001). Although cats can shed oocysts after ingesting oocysts, bradyzoites, and tachyzoites the efficiency of transmission varies. Virtually, all cats fed bradyzoites shed oocysts whereas less than half of cats fed oocysts or tachyzoites shed oocysts (Table 4). Additionally, the pp after ingesting oocysts or tachyzoites is long (18 days or more) and unpredictable (Table 4). Therefore, only the bradyzoite-induced cycle is known in cats. After the cat ingests bradyzoites, some bradyzoites multiply as tachyzoites in the lamina propria of the intestine and are disseminated in extra-intestinal tissues as in the intermediate hosts (mice). At the same time, some bradyzoites continue the enteroepithelial cycle giving rise to asexual stages (schizonts that contain merozoites) and merozoites give rise to sexual cycle (gamonts and oocysts) and the entire cycle can be completed in 3 days (Dubey and Frenkel, 1972). After ingesting oocysts and tachyzoites cats do not shed oocysts until 18 days and it has not been possible to find enteroepithelial stages of the parasite until oocysts have passed (18 days p.i), irrespective of the numbers of organisms fed to cats (Freyre et al., 1989; Dubey, 1996, 2002). It has been hypothesized that after being ingested by cats oocysts and tachyzoites first give rise to bradyzoites in tissue cysts, and when tissue cysts rupture, a few bradyzoites reach the intestinal mucosa to continue the enteroepithelial cycle and this event is unpredictable.

The *T. gondii* life cycle in cats and mice is not dose but stage dependent because few oocysts are non-infective to cats but one oocyst can be lethal for mice and a few bradyzoites (<10) are infective to cats but not to mice (Dubey, 1996, 2001). In mice fed bradyzoites or sporozoites, the organisms transformed to tachyzoites first and tachyzoites gave rise to bradyzoites in a period of 7–8 days (Dubey and Frenkel, 1976; Dubey, 1997; Dubey et al., 1997). Jerome et al. (1998) studied transformation of VEG strain sporozoites to tachyzoites and bradyzoites in cell culture. Sporozoites transformed to tachyzoites 24 h p.i. and multiplied as tachyzoites for 7–10 days when they began to accumulate bradyzoite-specific BAG1 antigen. However, in cultures that were stressed (with high pH in the medium) some sporozoites transformed directly to BAG1 positive organisms. In this respect, the data obtained in the present study by inoculating oocysts (sporozoites) in to mice peritoneum and 5–7 days later feeding the resulting organisms to cats are noteworthy. With the TgCkAr23 the minimum prepatent period was 15 days and the pex was removed 7 days p.i. but with the VEG strain the minimum period was only 10 days and the pex was removed 5 days p.i. (Tables 1 and 2). These data suggest that sporozoites had transformed to functional bradyzoites as soon as 5 days p.i., but do not lend support to the hypothesis that sporozoites can transform to bradyzoites directly because 5 days would allow sporozoites to convert to tachyzoites (1 day) and tachyzoite to bradyzoite (3–4) days (Dubey and Frenkel, 1976). I considered removing pex from mice 1–4 days p.i. and then feeding to cats but did not do so because pex removed at 7 days p.i. (Table 2) after injecting sporozoites of the TgCkAr23 isolate gave rise to long pp.

In the present study, more than 60% of cats administered *T. gondii* from the pex of mice became infected, irrespective of whether the inocula contained organisms that gave rise to oocysts with a short pp (meaning inocula contained bradyzoites) or long pp (meaning that inocula contained tachyzoites). These results were also independent of the mode of administration. It is likely that some *T. gondii* might penetrate pharyngeal-buccal mucosa when *T. gondii* was poured in the mouth of the cat. However, 17 of 31 cats administered *T. gondii* in pex by a stomach tube also became infected indicating that they survived acid-pepsin digestion in the stomach. These results confirm our earlier observations concerning the survival of tachyzoites in acid-pepsin (Dubey, 1998a). These results indicate that humans can become infected if they accidentally ingest *T. gondii* tachyzoites in the laboratory.

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